

CYTOGENETICAL INVESTIGATIONS ON SPIDERS OF SEMI-ARID AREAS

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ABSTRACT

Spider species have always been avoided for cytogenetical examination largely because of its structure and size and the tedious process of obtaining the chromosome complements. Only 792 species and 288 genera of spiders are cytogenetically known worldwide. In India, the western ghats and some north eastern species have been explored, however, cytogenetical database studies for spiders from semi-arid areas are scanty. The traditional methods for chromosomes preparation by air dry/flame dry methods required dissection of gonads and other tissues. In the present study somatic cell culture approach has been made on six spiders species (*Crossopriza lyoni*, *Menemerus sp.*, *Cyrtophora sp.*, *Selenops sp.*, *Hersilia savignyi* and *Neoscona theisi*) with success. The best chromosome plates obtained at metaphase stage from each spider species exhibited diploid 2n ranging from *C. lyoni* (24), *Neoscona theisi* (24), *Cyrtophora sp.* (26), *Menemerus sp.* (28), *Selenops sp.* (29) and *Hersilia savignyi* (30). The karyometrical analysis system reveals three categories (macro as in 1-5 pair *C. lyoni*, semi macro 6-10 pair as in *Selenops sp.* and micro 9-13 pairs as in *Menemerus sp.*). The sex chromosome mechanism varied from XX (*C. lyoni*), X1X2 (*Cyrtophora sp.*, *Menemerus sp.*, *Neoscona theisi* and *Hersilia savignyi*) to X1X2X3 (*Selenops sp.*). The current paper proposes Somatic Cell Culture as a rapid and cost effective short term result oriented technique for cytogenetical analysis of spiders (Araneae) and discusses the karyometrical data base of spiders of semi – arid areas of India.

Key words- Spiders, Somatic cell culture, cytogenetics, karyometry, semi-arid areas, Agra, India.

INTRODUCTION

Among the invertebrates, the spiders (Araneae) are highly diverse group of fauna. A total of 45,122 species and 3932 genera are known (World spider catalog, 2015) out of which about 1687 species belonging to 438 genera and 60 families are reported from India (Keswani *et al.*, 2012). The spider diversity in semi-arid agro-ecosystems is currently under survey (Anjali and Prakash, 2012). The knowledge on cytogenetics of spiders is scanty only 792 species and 288 genera are

karyometrically known. Cytogenetic studies have been undertaken as a part of genetic diversity of spiders from agro-ecosystems of semi-arid area to build a database of 2n karyomorphology, and sex determining mechanisms of six species of spiders from different families and also established inter-relationship within the members of the cytogenetically known species.

The chromosome number of spiders varies from $2n = 7 \sim 110$ and the species exhibit multiple sex determining chromosome system (Araujo *et al.*, 2012). Various attempts have been made for searching cost effective and rapid methods for obtaining quality chromosome complement and this study is a step towards overcoming the difficulties in karyology of spiders by somatic cell culture.

MATERIALS AND METHODS

Spider collection and identification- Spiders were collected from different sampling sites by hand collection and visual searching methods (Fig. 1) Identification was done by following different keys available on web and books (Platnick 2014, Sebastian and Peter 2009, Tikader 1987 and Siliwal 2005).

Collection of tissue- Live spiders were washed with double distilled autoclaved water in watch glass then the legs were gently separated from the body. The body was crushed with fine clean blade and the clear body fluid was collected through micropipette and transferred to culture media.

Cell Culture - Initially following media were tried for cell growth

1. *Drosophila* Schneider medium
2. Hikaryol XLTM RPMI culture medium

The protocols followed for cell culture and chromosome preparations have been described earlier by Anjali and Prakash (2014), Onrat *et al.*, (2007). The position of centromere was determined according to Levan *et al.*, (1964)

Following cytogenetic parameters were measured:

1. Arm ratio or $r = \frac{\text{Length of the long arm}}{\text{Length of the short arm}}$
2. Total length of a chromosome [TLC].
3. Centromeric position and chromosome type.
4. Sex determining chromosome system (SDCS)

Nucleolar organizing regions (NORs)

The single step method for silver staining was used with suitable modification (Howell and Black, 1980; Prakash, 1996).

RESULTS

Several methods for preparing chromosomes from adult spiders were tried which included the standard thumb- squash method, the flame dry method and the air dry method with little success in getting chromosome compliments of good quality. A direct dissection of gonads for dividing cells is an extremely tedious procedure (Webb *et al.*, 1978), therefore, became imperative to innovate a better and a faster method. Since the mammalian cells are routinely cultured for chromosome preparations, we made attempts to perform short term somatic cell culture (SCC) (Fig. 2) and followed it with the standard air dry methods. The successful cell culture gave good harvest of metaphase plates. Male and female adults of six species *C. lyoni* (Blackwall, 1867); *Hersilia savignyi* (Lucas, 1836) ; *Neoscona theisi* (Simon, 1864); *Selenops sp.*(Latreille, 1819); *Menemerus sp.* (Simon, 1868) and *Cyrtophora sp.*(Simon, 1864) (Fig. 18) were subjected to SCC and the results are as follows.

C.lyoni (Blackwall, 1867) is the predominantly abundant spider in Agra and is highly adaptive to semi-arid habitat. The karyological analysis of oogonial metaphase depicted $2n=24 [22+XX]$. The chromosomes at oogonial metaphase stage appeared to be highly condensed and rod shaped with both sub-metacentric and metacentric orientation .The karyotype can be categorized under two groups, metacentric (10) sub metacentric (14). The autosomal chromosomes gradually decreased in size (Fig. 3). The maximum chromosomal length reported was (TLC- 0.9 cm) (Fig. 4). In the diplotene and diakinesis stage of male *C. lyoni*, the X chromosome appeared as extremely large and isopicnotic in behavior. In these cells the most of the bivalent presents the interstitial chiasma with the cross configuration assuming a ring like structure. The sex determining chromosomal system was predominantly and characteristically XO and XX system in *C. lyoni*.

The silver stained metaphase NOR revealed three paired nuclei in female. Attempts to resolve the number of NOR in females in the chromosome itself was unsuccessful, however, in the interphasic stage the micronuclei in female could be located. The metaphase plates of males and females both revealed a NOR pair in the proximal region of the short arm of the 6th pair (Fig. 16). Existence of a single strongly stained block in the micro-chromosome, could not be assigned to a cytogenetic value, however, they may represent a particular sex chromosome as recorded (Oliveira *et al.*, 2007)

Hersilia savignyi (Lucas, 1836) is a foliage runner which has two tailed spinnerets and is commonly found in this region. The female is 7-10 mm long and males are shorter as compared to the females. The Cytogenetical analysis showed the diploid number $2n= [28+X1X2]$ (Fig. 5).The chromosomes in females at metaphase stage were observed in four groups, that is metacentric (22), sub-



Figure 1- Study area map

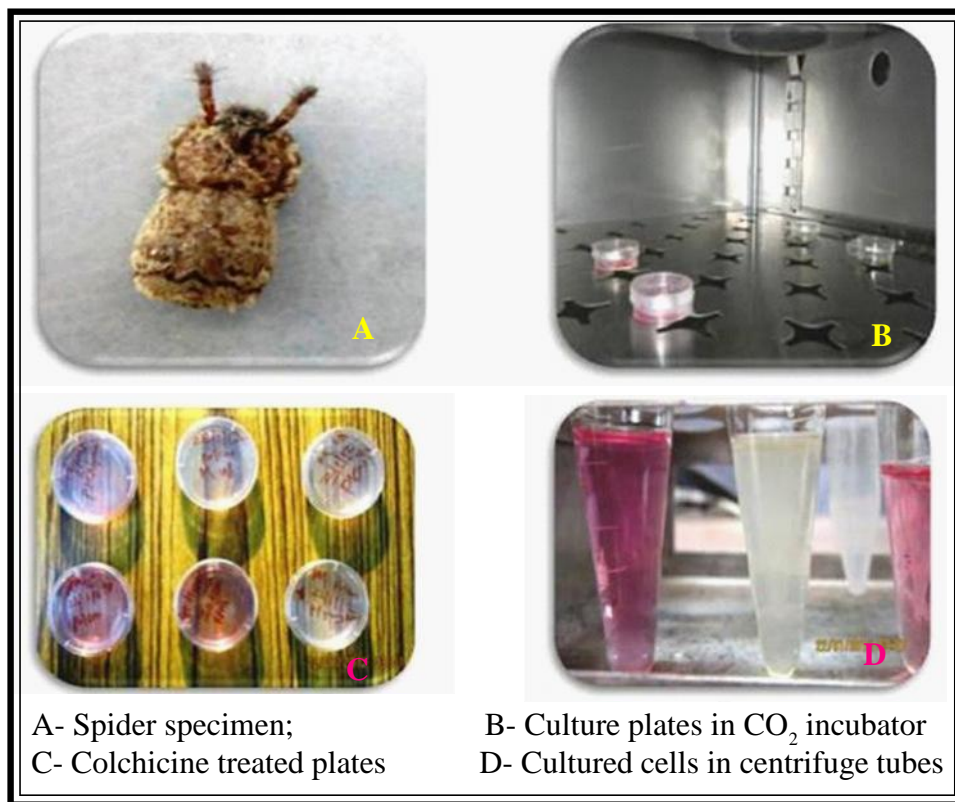


Figure 2- Steps of Somatic Cell Culture

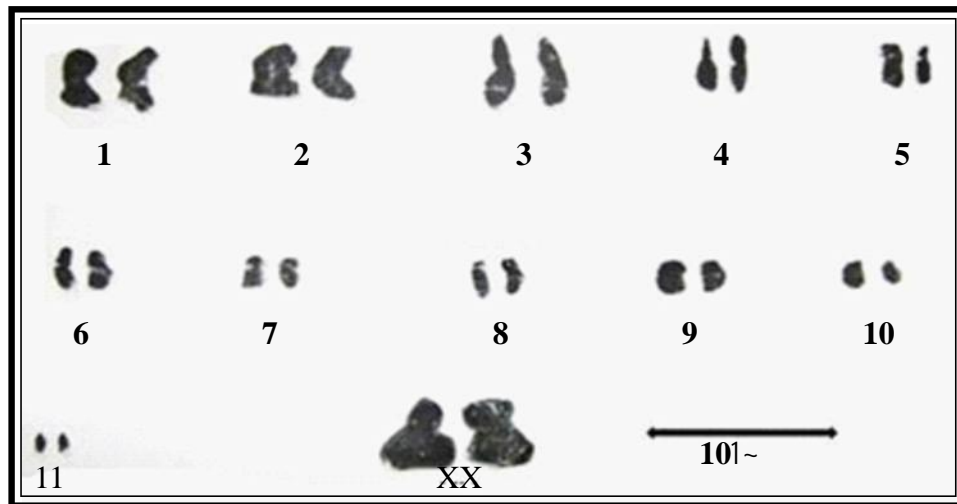


Figure 3- *C. lyoni* Karyotype

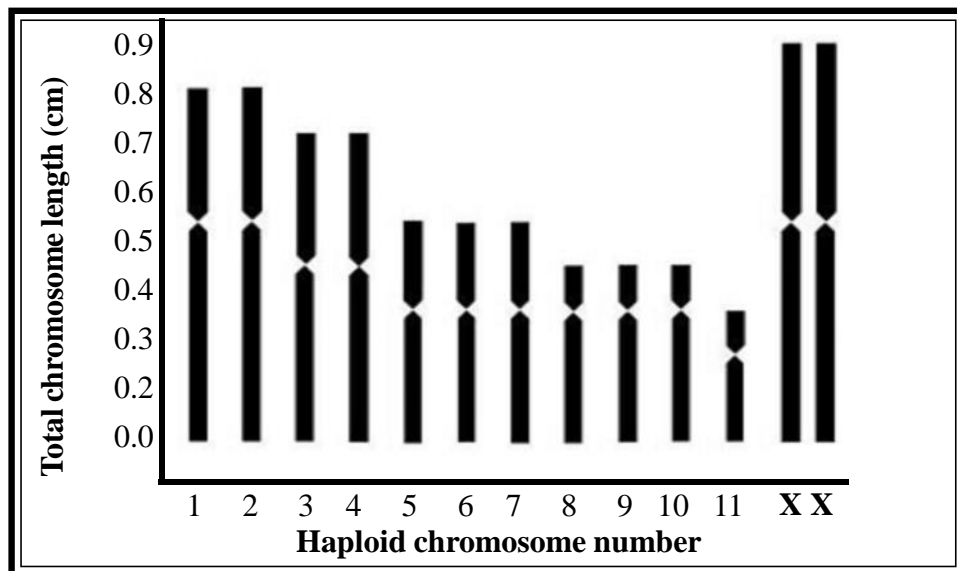


Figure 4 - Ideogram of *C. lyoni*

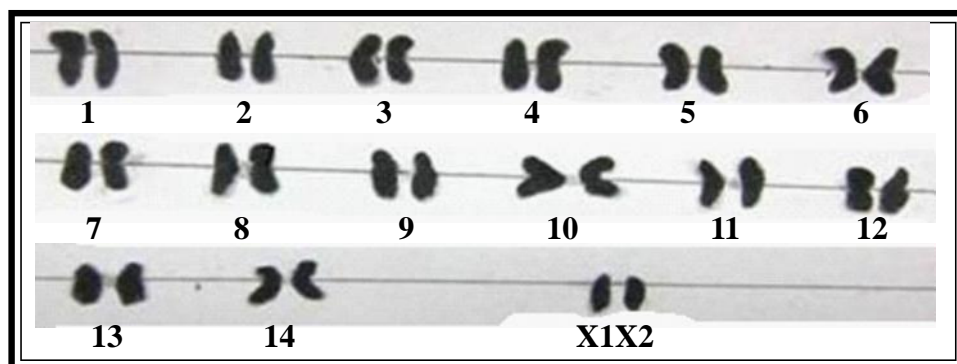


Figure 5- *Hersilia savignyi* Karyotype

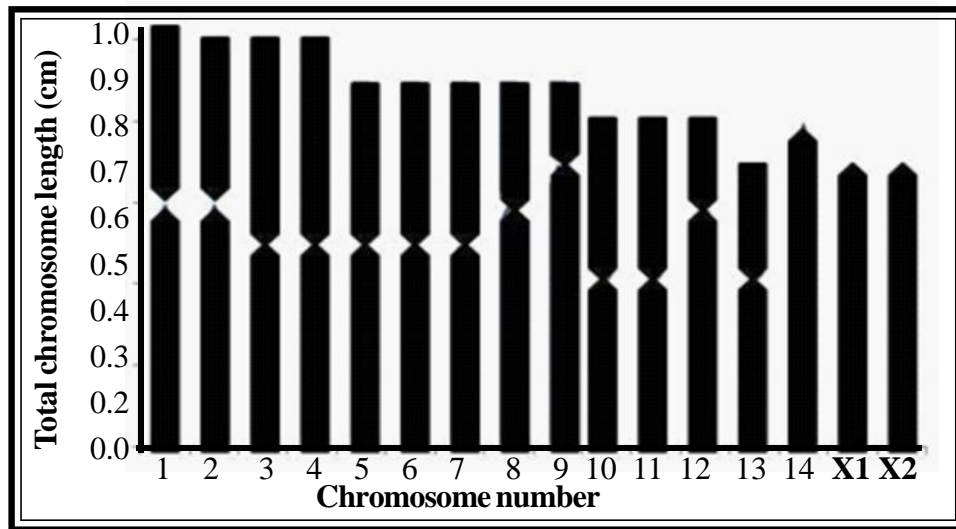


Figure 6- Ideogram of *Hersilia savignyi*

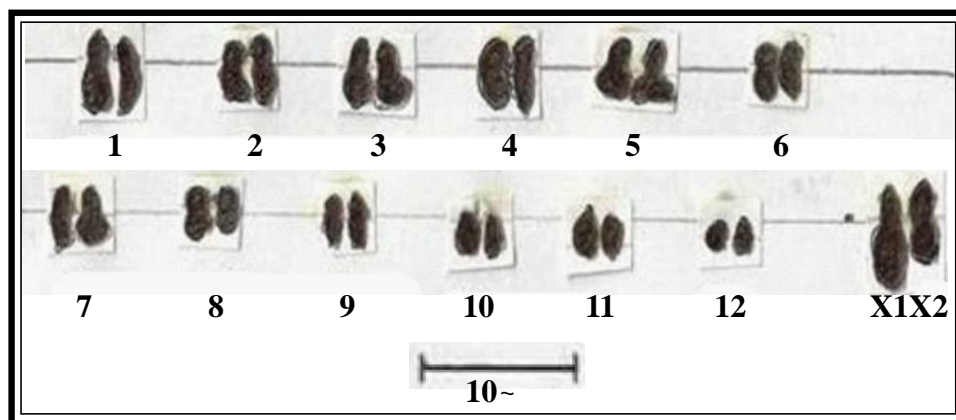


Figure 7- *Cyrtophora sp.* Karyotype

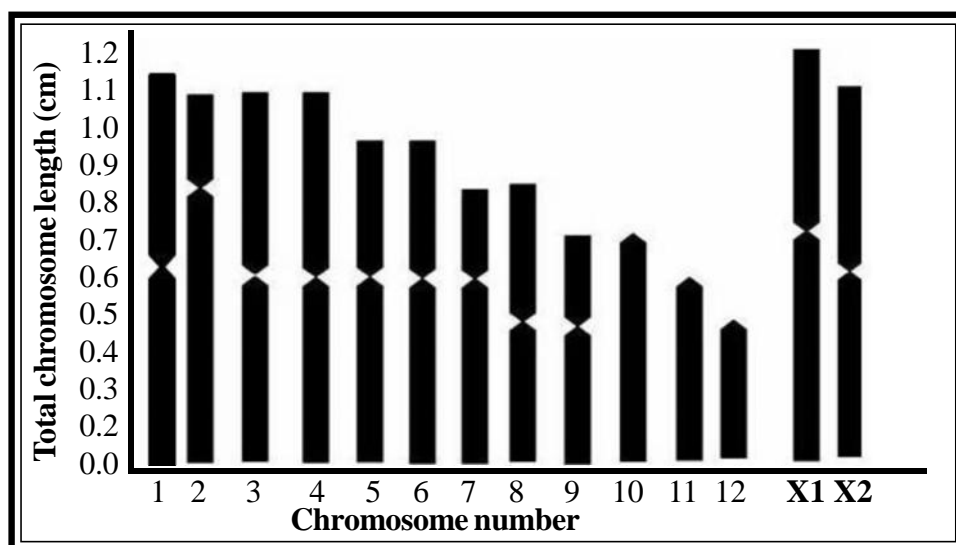


Figure 8- Ideogram of *Cyrtophora sp.*

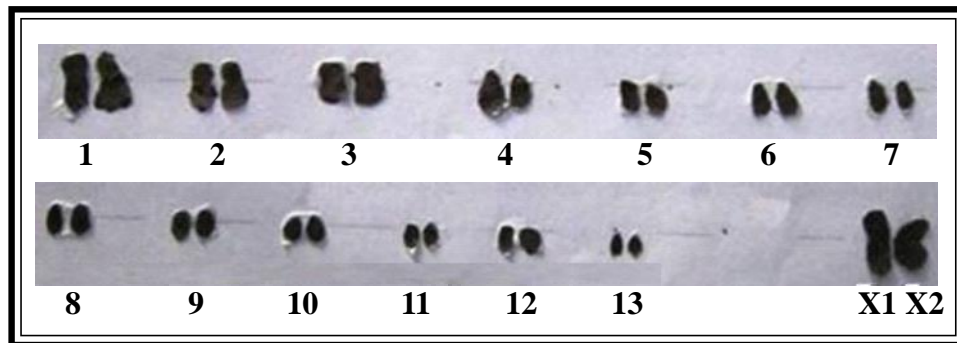


Figure 9- *Menemerus* sp. Karyotype

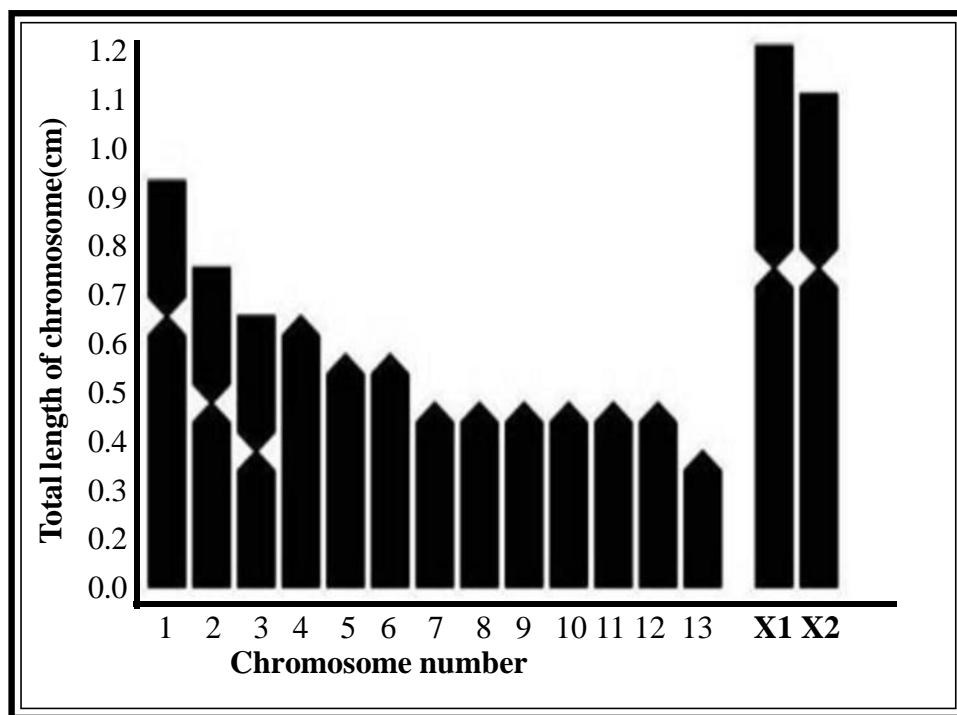


Figure 10- Ideogram of *Menemerus* sp.

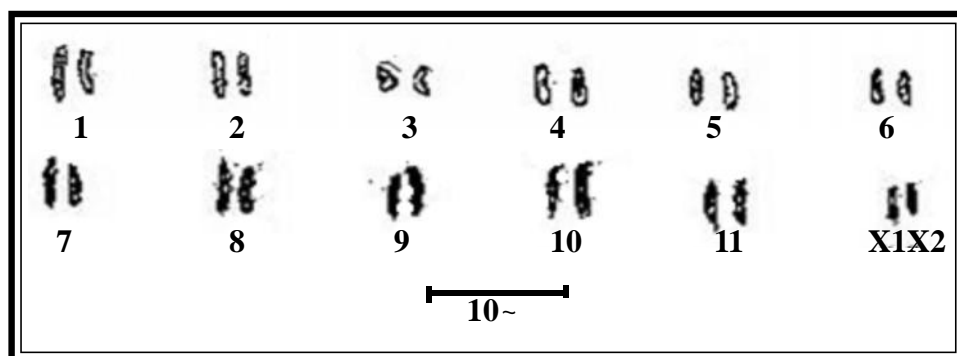


Figure 11- *Neoscona theisi* Karyotype

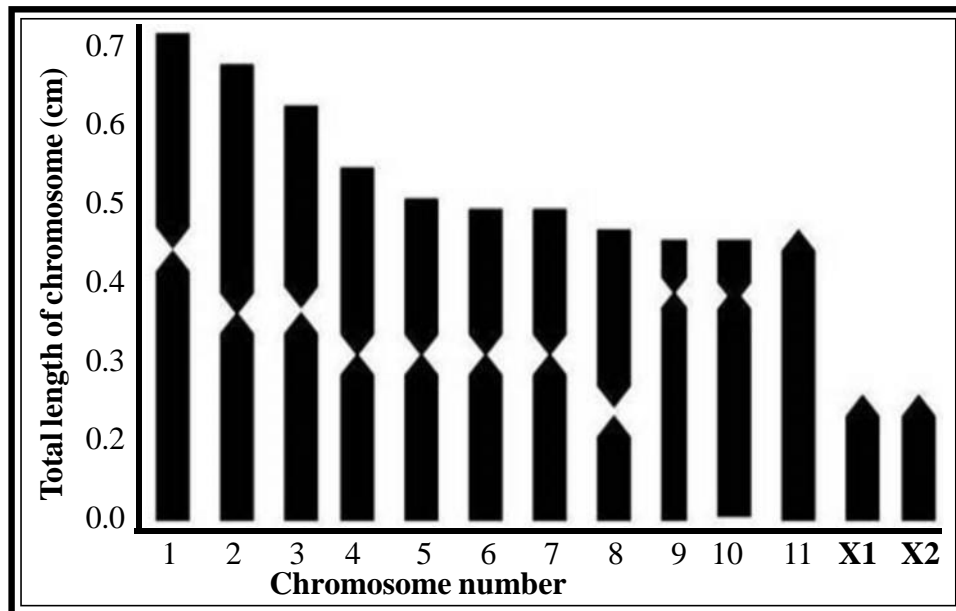


Figure 12- Ideogram of *Neoscona theisi*

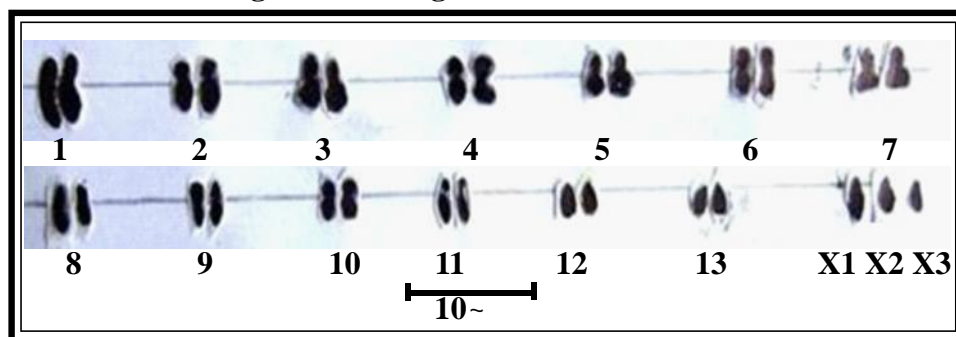


Figure13- *Selenops sp.* Karyotype

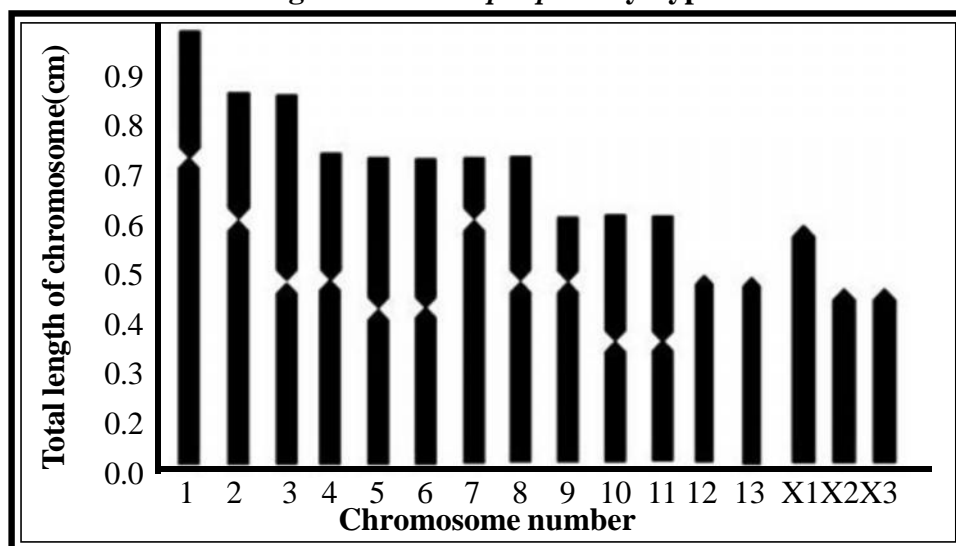


Figure 14- Ideogram of *Selenops sp.*

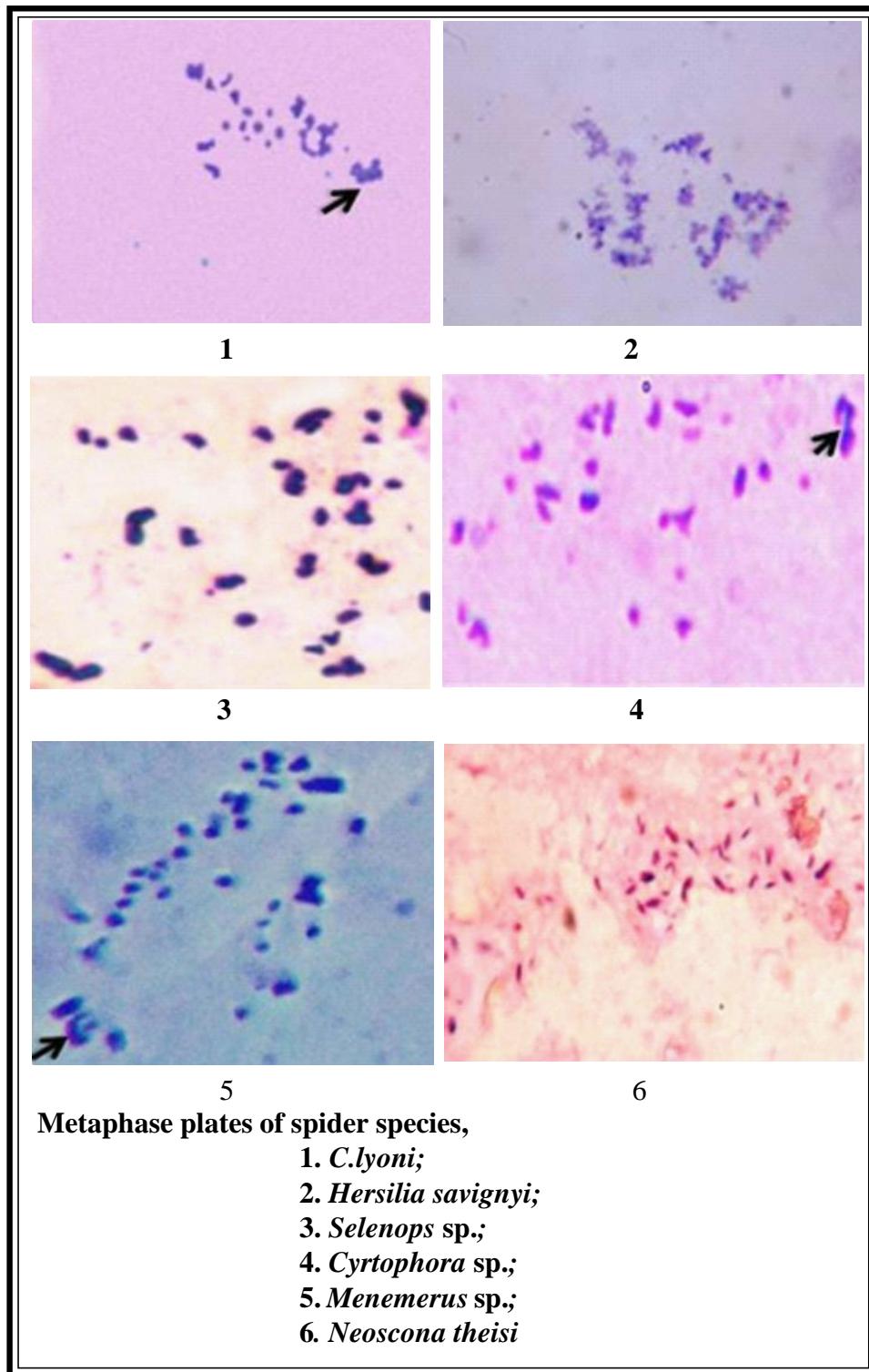


Figure 15- Metaphase plates of spiders showing multiple sex determining chromosome system (SDCS)

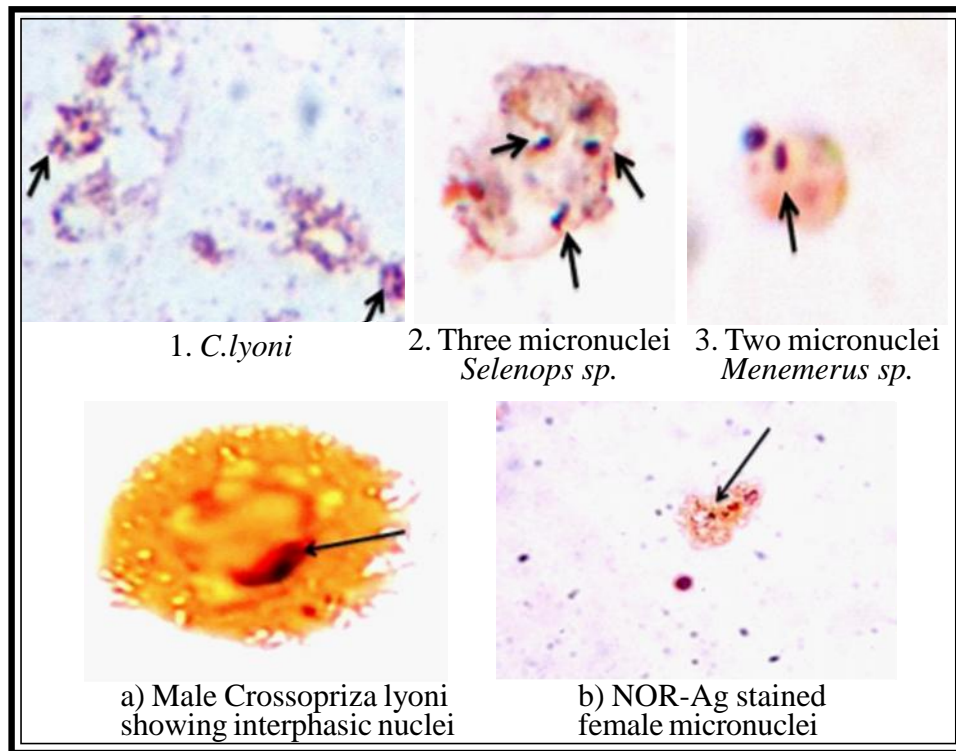


Figure 16- NORs localization in spiders

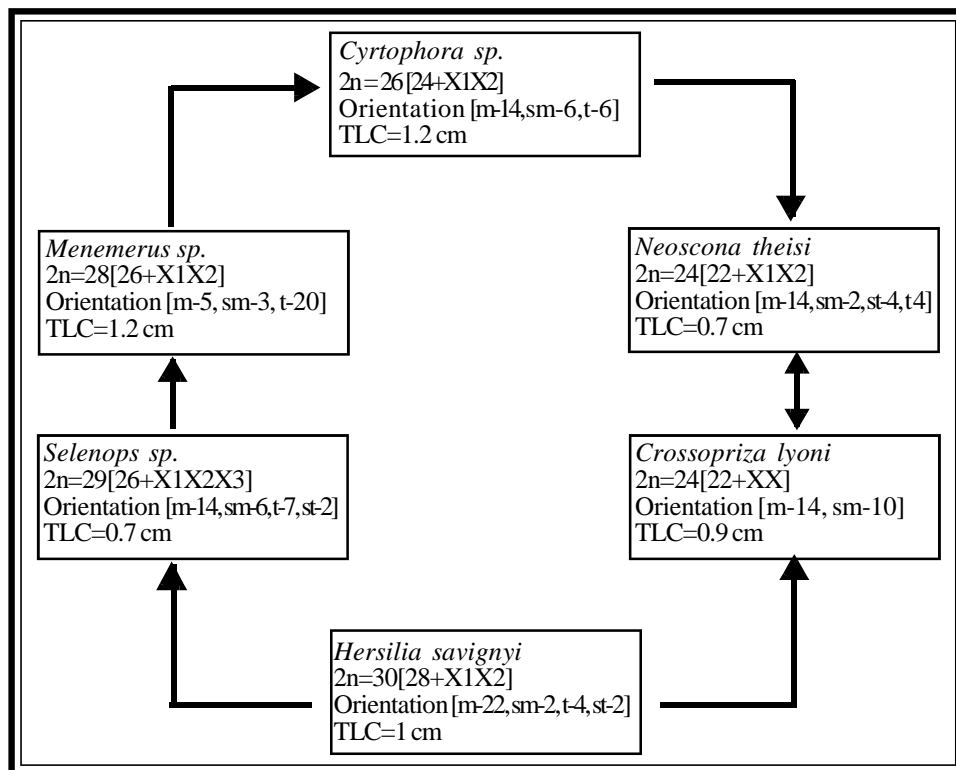


Figure -17 Cytogenetic Model for spiders of semi-arid areas

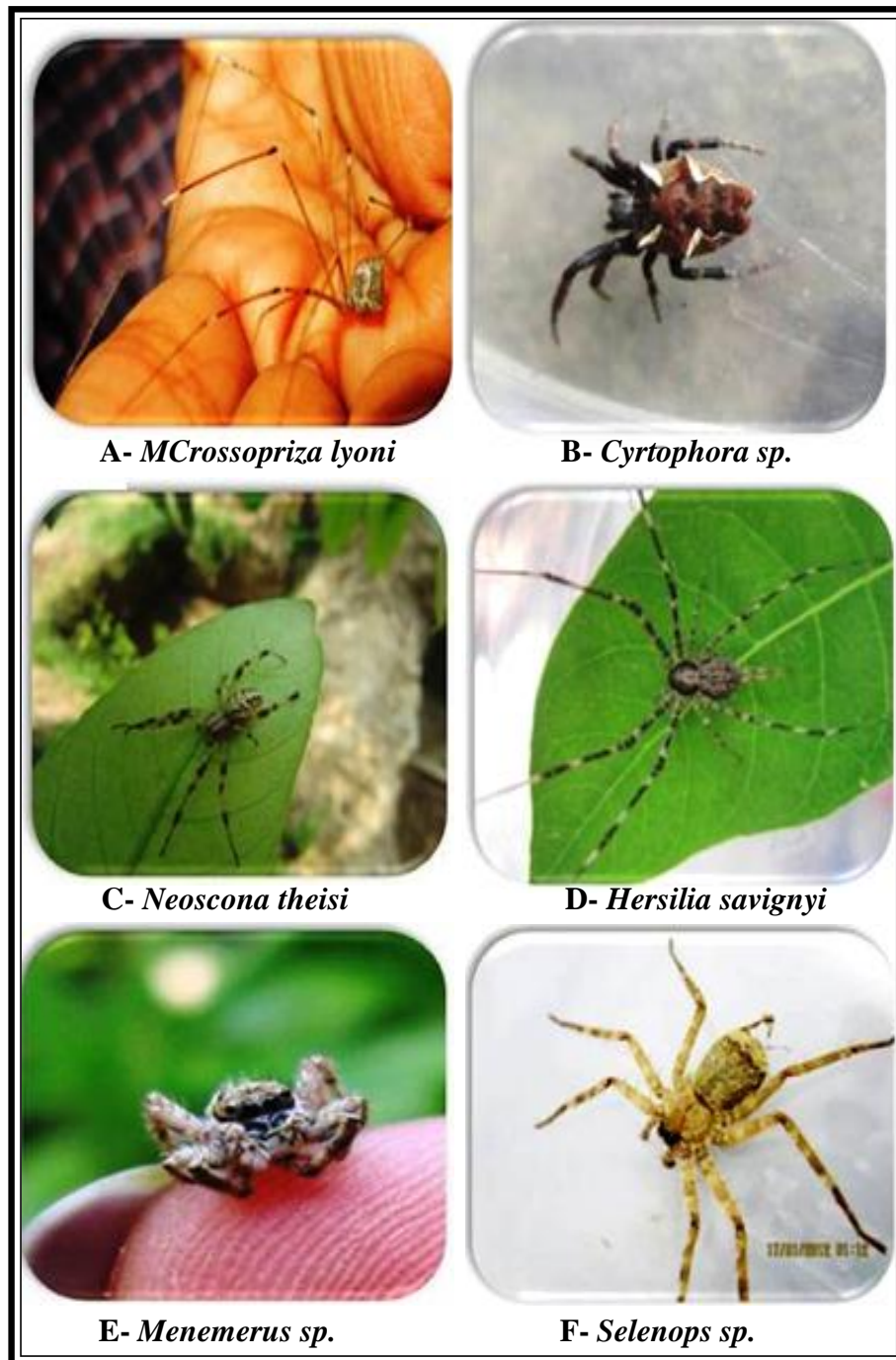


Figure 18 - Spiders of Semi-arid areas of Agra

metacentric(2) , telocentric(4) and Sub-telocentric(2). The 28 autosomes were decreased in size. The TLC value was 1 cm. (Fig 6.). In the Diakinesis stage the sex chromosomes were heavily stained and the SDCS was X1X2 type with telocentric orientation. Silver stained micro nucleoli were recorded which may indicate the X chromosome.

Cyrtophora sp. (Simon 1864) is the third targeted species from this area. This spider is commonly observed on babul and berry plants which are highly adapted to semi arid habitat. The karyometrical data shows the spermatogonial diploid number $2n=26$ ($24+X1X2$) (Fig.7). The maximum total length of chromosome was 1.2 cm with sub metacentric orientation. The autosomes were mainly of three types metacentric (14), sub-metacentric (6) and telocentric (6). Heteropycnotic bodies were observed which helped to make individual forms in diakinesis stage. Premeiotic interphasic nuclei revealed highly condensed heteropycnotic elements during the diakinesis and diplotene stage. The chromosomes were dispersed and the sex chromosome system was $X1X2$ with sub-metacentric orientation and with largest TLC value (Fig 8). Ag-NORs stained micro-nucleoli with two heteropycnotic block may correspond to SDCS (Fig 16).

Neoscona theisi (Simon, 1864) is a garden spider. Its karyological details showed the spermatogonial metaphase count diploid number of $2n=24[22+X1X2]$ from many well-spread nuclei. The TLC was 0.7 cm. The autosomes were categorized in metacentric(14), sm(2), st (4) and telocentric(4) (Fig. 11). The SCDS $X1X2$ type is with telocentric orientation (Fig. 12).

Menemerus sp. (Simon, 1868) is a jumping spider which belongs to family Salticidae. The karyotype showed $2n=28[26+X1X2]$ (Fig.9). The autosomes were sub-metacentric (3), metacentric (5) and telocentric (20). The sex chromosome were heavily stained with maximum TLC value (1.2 cm) (Fig 10). Ag-N03 stained micronucleoli were observed.

Selenops sp. (Latreille, 1819) belongs to the family Selenopidae. It is a ground dweller spider found on shady and moisture places. The total length of chromosome is 0.7 cm (Fig 14). This is also visualized as three micro nuclei during silver staining of NORs , autosomes were sm(6), m(14),st (2) and telocentric (7) (Fig. 13). The sex chromosome system was observed as $X1X2X3$ with telocentric orientation.

DISCUSSION

This study made an attempt for the first time to describe the chromosome details and diversity of chromosome from six species of spiders using “short term somatic cell culture” method. The results obtained have been rapid and cost effective using limited number of spiders. The aim of this study was to resolve the cytogenetical profile of spiders *visa-a-vis* genetic diversity among the spiders of agro-ecosystems which would be beneficial to biologist attempting to conserve spiders.

The diploid chromosome number in spiders ranged from $2n=24\sim30$ (Fig. 15). The species from Pholcidae family represented the lowest chromosome number ranging between $15\sim24$ and the maximum number is obtained from the family Hersilidae ($2n=30$). The majority of Indian spider species cytogenetically known

are from rain forest and western ghats region (Datta and Chatterjee, 1988). The species in these areas have generated a wide range of chromosome diploid number, thereby creating difficulty in assigning a single characteristic diploid number to the species. A case in point is *C. lyoni* which has been found a fix frequency number $2n = 24$ in the agro-ecosystems of Agra, this number is also found in the wide range of $2n$ numbers from western ghats. therefore, the present study is a possible confirmation and affirmation of the predominant $2n$ number of *C. lyoni*. Similarly the *Cyrtophora sp.*, *Hersilia savignyi*, *Selenops sp.* and *Neoscona theisi*. was confirmed (Araujo *et al.*, 2014).

Thus in the present studies the chromosomes expressed all the four orientations in their karyotype-(sm, m, t and st), however, a single species expressed maximum four types of orientation. The chromosomes appear highly condensed making it difficult in identifying the univalent and bivalent at diplotene/diakinesis/metaphase stage, however, the X chromosomes appears to be the largest and their reduction in $2n$ can be interpreted as centric fusion involving one pair of X. Multiple sex chromosome systems exists in spiders. Most common sex determining X1 X2 male and X1X2X1X2 in female apart from these some system also show X1X2X3 in females (Parida and Sharma 1987, Srivastava and Shukla, 1986) The X1X2O system is predominant and therefore presumed to be the precursor for X1X2X3 system that is the their X is originated from X1 or X2 through tandem or centric fusion as suggested by Suzuki (1954) while one of the three XXX in X1X2X3 may represent one of the X is in the X1X2 species, the other X originated from the remaining one by a complicated crosses of miss division of centromere, followed by inversion resulting in a dicentric X chromosome" therefore it may be reduce that X1X2X3O system in male spider one of the X will always be largest size than the other two (Araujo *et al.*, 2012) this has been observed in Araneidae and Selenopidae (fig. 15). Spiders have been also classified depending on their chromosome number as primitive or advanced system (Datta and Chatterjee, 1988). The extremely high chromosome numbers have been considered as primitive while the chromosome number 18~24 are transact type while the less than 15 are considered to be recent. In this process 3 genus under our investigations can be grouped under intermediate type and *Hersilia savignyi*, *Selenops sp.*, *Menemerus sp.*, *Cyrtophora sp.* are relatively primitive to the *C. lyoni* and *Neoscona theisi* (Fig. 17). Heteropycnotic behavior of sex chromosome indicate low/insignificant heterochromatinised nature, a fact substantiated by the failure of obtaining good C-banding bands.

The nucleolus organizer regions (NORs) are the sites of the 18S + 28S rDNA, which indicate the presence and the position of the secondary constriction in the chromosome of the species and become important characteristics in the karyosystematics. The NORs can be easily determined in metaphase chromosome by a quick and simple staining of silver nitrate (Howell and Black, 1980) which appears to stain nucleolar phospho-protein B23 and C23 associated with rDNA

transcription (Schwarzachr and Wachtler, 1983). It is the specificity of silver staining for complex gene locus that makes NOR potentially useful as chromosome marker for Cytogenetics and Cytotaxonomic studies. Intensively silver-Ag stained regions of the chromosomes correspond to secondary constriction as a rule. Though we consistently found only one pair of the Ag-NORs in the 6th pair of the *C. lyoni* but presence of micro nucleoli at interphase indicate the sex chromosome linkage. Secondary NORs cannot be excluded with absolute certainty because additional blocks of positively stained silver were also located in many species. In general the NORs in spiders is very conservative and that is why condensed, however, the recent one i.e. *C.lyoni* show clear presence and positions the secondary constrictions which are potentially NORs. Similar results have reported in other Pholcids (Oliveria *et al.*, 2007). Occurrence of specific X chromosomes NORs has been observed in *Selenops sp.* occupying the short arm. The species were bearing X1X2X3 sex determining system type (Fig.16)

ACKNOWLEDGMENTS

I would like to thank Dr. Manju Siliwal for her comments on identification of spiders. I am also thankful to the UGC (RGNFS) for providing financial support.

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